Blood Substitutes

Where are we now??

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What am I talking about?

Hemoglobin and Hemoglobin Substitutes

Goals

- Explain why we need this
- Review hemoglobin physiology
- Detail the history of blood substitutes
- Discuss current hemoglobin based oxygen carriers

Why is this needed??

Limitations of heterologous blood

- Limited availability of blood
- Compatibility issues
- Storage limitations
- Disease Transmission
- Immunomodulation

Case Report—Denver Health Medical Center

- 18 yo male GSW to abdomen 30.06 220 grain hollow point bullet
- Transfused 10 units Polyheme during 1st 14 minutes of resuscitation
- Polyheme represented 91% of circulating hemoglobin
- Injuries found at laparatomy—shattered kidney, aortic and IVC perforations, spinal cord transection, injuries to all sections of bowel.
- Total transfusion 40 units over hospitalization
- Survived to discharge without organ dysfunction

Terrorism

- World Trade Center and Pentagon
  - >3,000 deaths
  - Extremely lethal: killed to wounded ratio 5:1
- Oklahoma City
  - 167 deaths
  - More typical killed to wounded ratio of 1:5

- **Do you want to be in charge of the triage with our current blood supply**

Dispersed Battlefield

- Evacuation times are related to mortality
- 20% mortality with evacuation in less than 2 hours
- 26% at 6 hours
- 32% at 24 hours *Bellamy, Mil Med 1984*

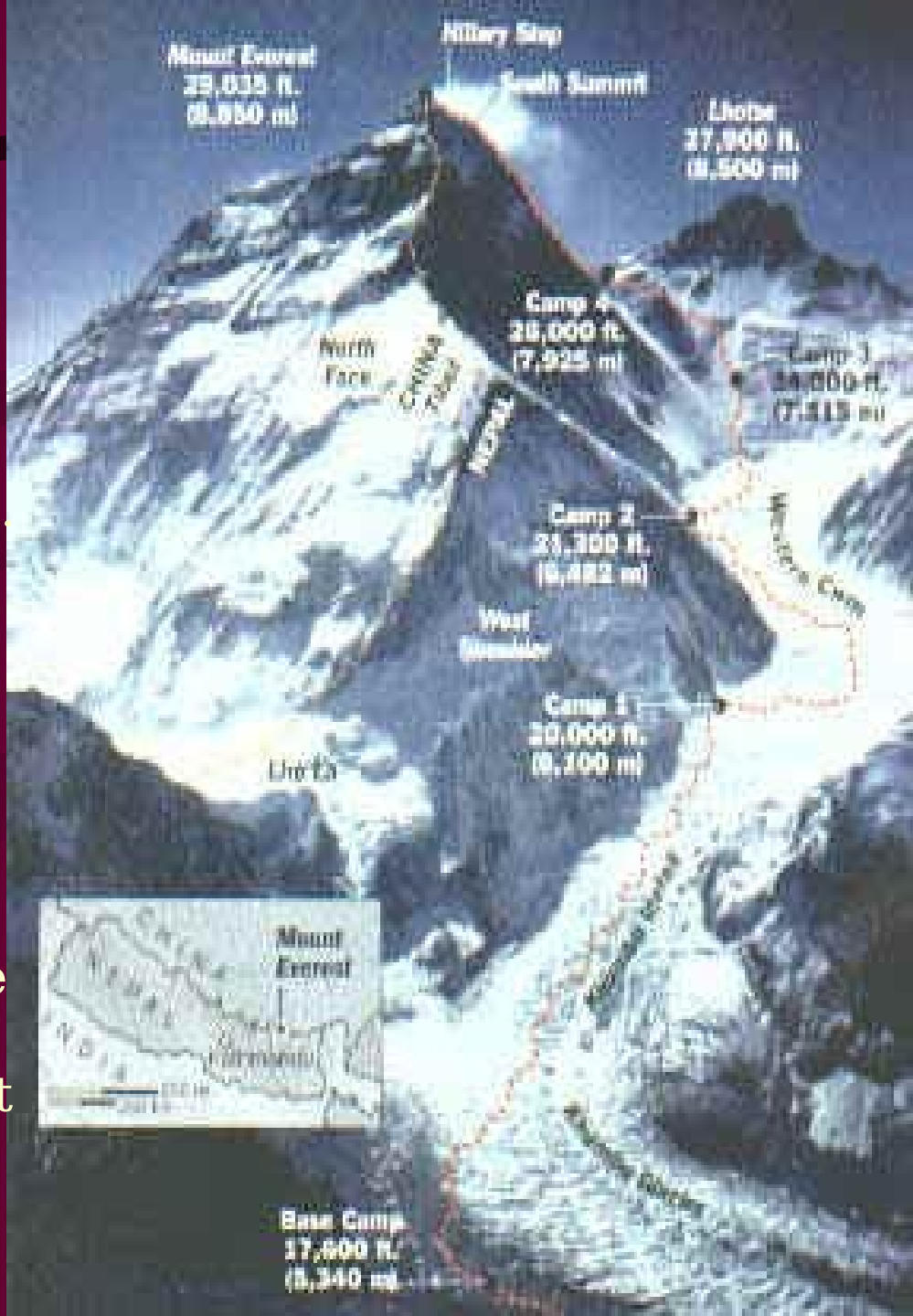
Case Report

- 39 y/o female Jehovah's witness
  - 4 children
- Presented with abruptio placenta
  - HB 2gm/dl, HR>135, ST segment depressions, Fetal demise
- Transfused 18 units of PolyHeme and underwent hysterectomy
- Survived to discharge

Hemoglobin Physiology

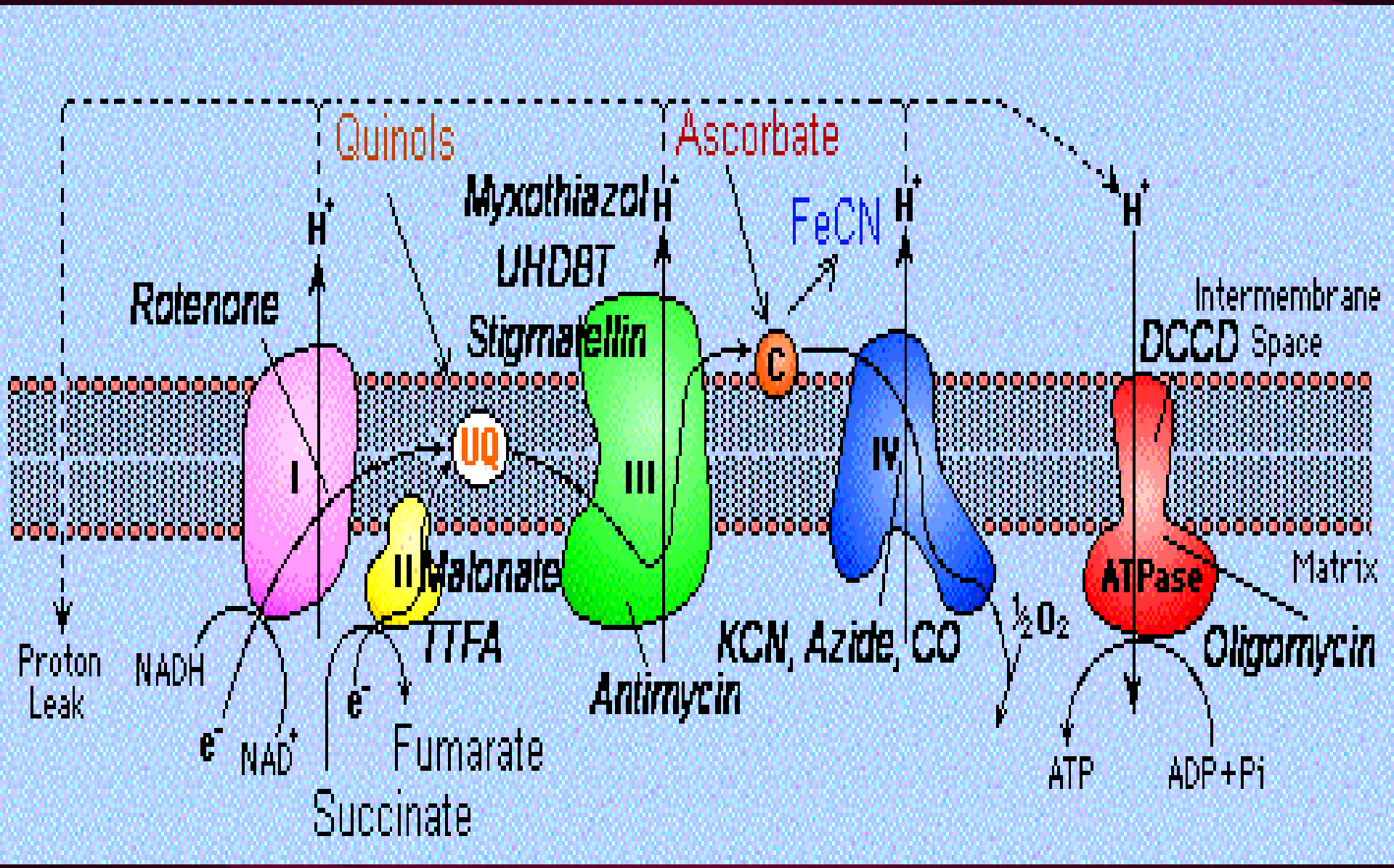
Wanting to conserve whatever oxygen remained in my tank, I asked him to reach inside my backpack and turn off the valve on my regulator, which he did. For the next ten minutes I felt surprisingly good. My head cleared. I actually seemed less tired than with the gas turned on. Then, abruptly, I felt like I was suffocating. My vision dimmed and my head began to spin. I was on the brink of losing consciousness. Instead of turning my oxygen off, Harris, in his hypoxically impaired state, had mistakenly cranked the valve open to full flow, draining the tank. I'd just squandered the last of my gas going nowhere.

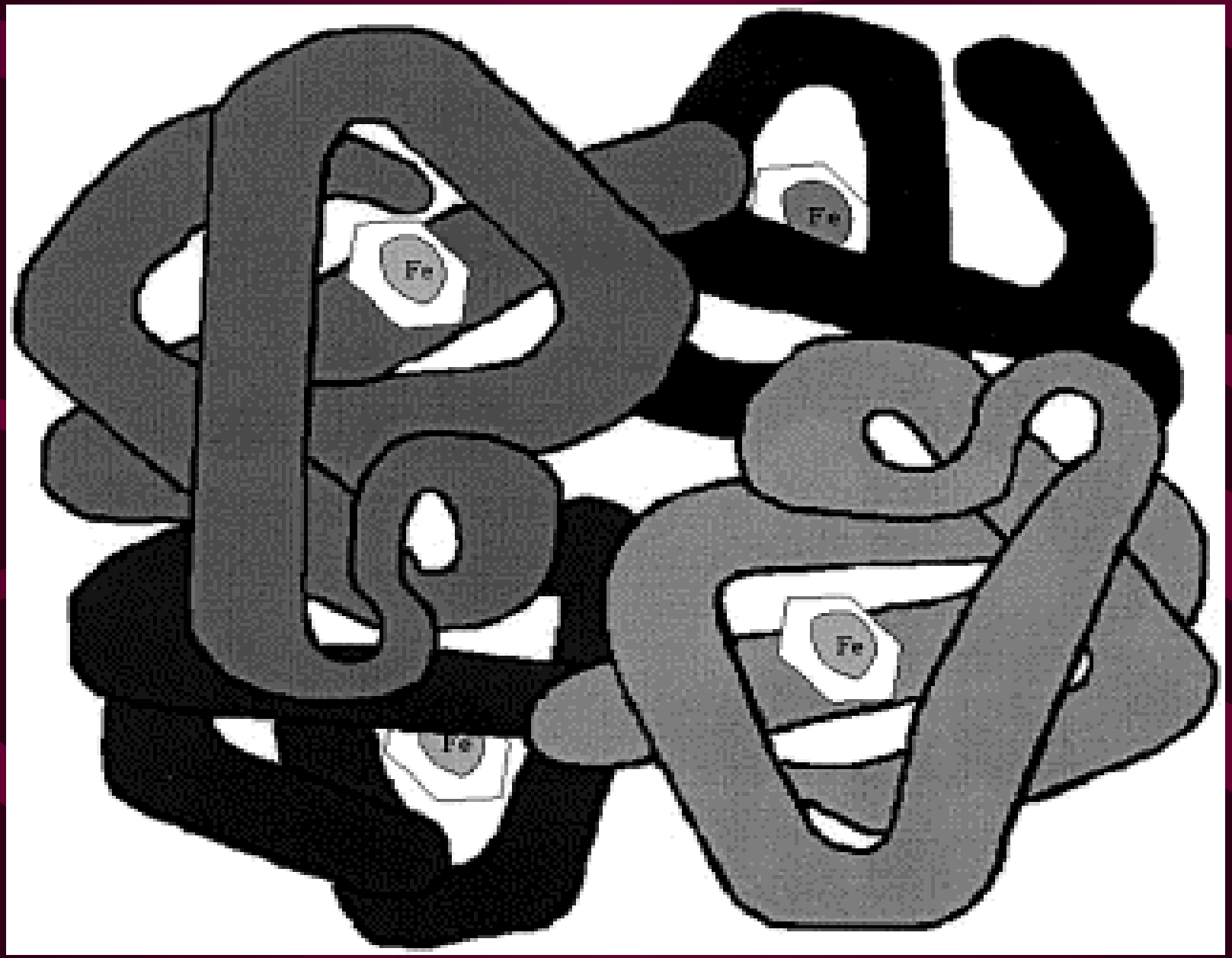
-John Krakauer, near the summit of Mount Everest



It’s all about oxygen delivery

$$DO_2 = CO * 1.34(Hb * sat) + 0.003 * paO_2$$


Quinols
Ascorbate
Myxothiazol
UHDBT
Stigmatellin
FeCN
Rotenone
Intermembrane
DCCD
Space
UQ
C
I
III
IV
II
Malonate
ATPase
Matrix
TTFA
KCN, Azide, CO
½O2
Oligomycin
Proton
Leak
NADH
Antimycin
Fumarate
Succinate
ATP
ADP+Pi
H+
e-
NAD+


Fe
Fe
Fe
Fe

Deoxyhemoglobin

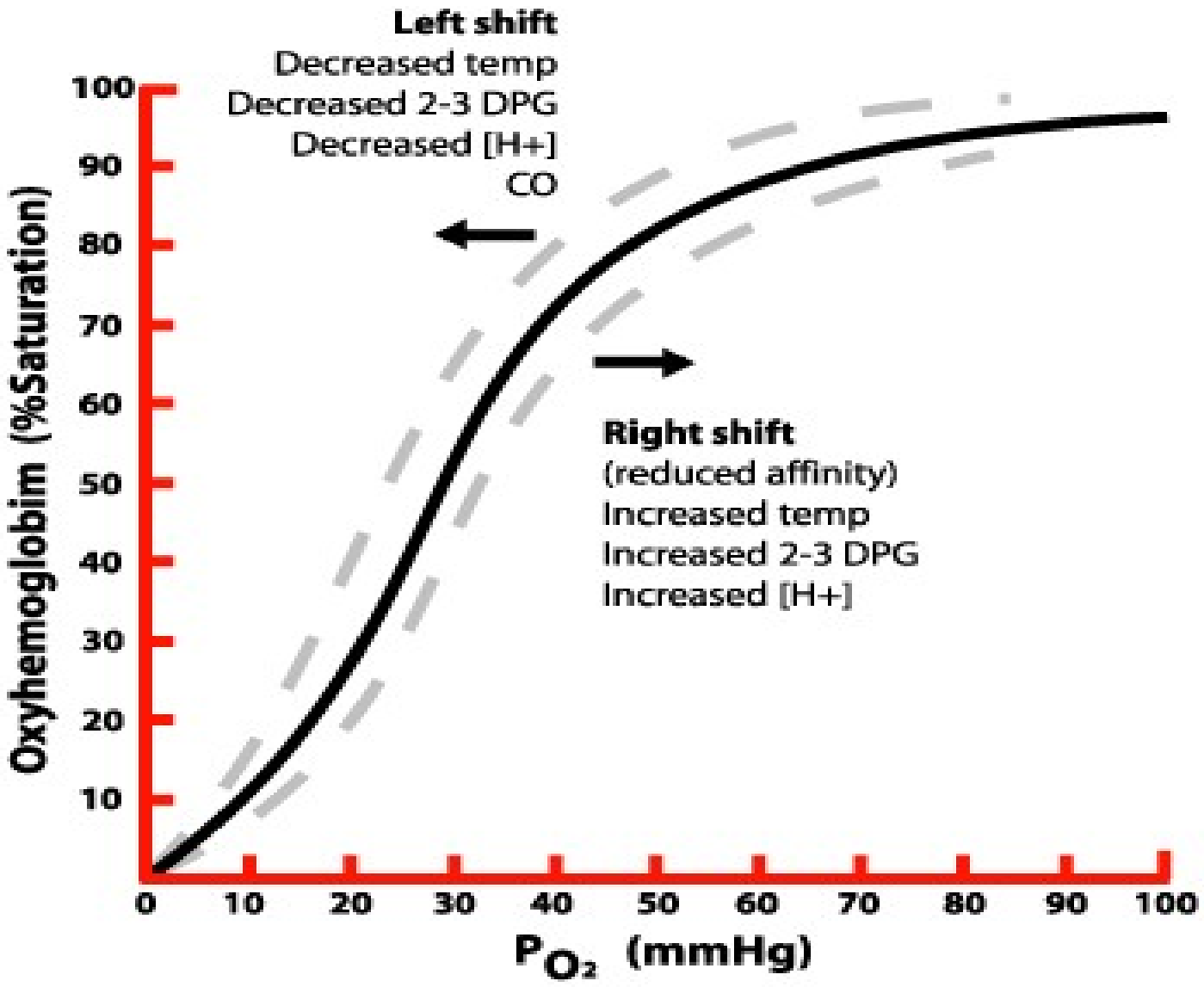
- Globin subunits are held in a tense configuration by electrostatic forces
- Low affinity for oxygen
- When oxygen binds a heme group the electrostatic forces weaken and a more relaxed configuration results

Hill Coefficient

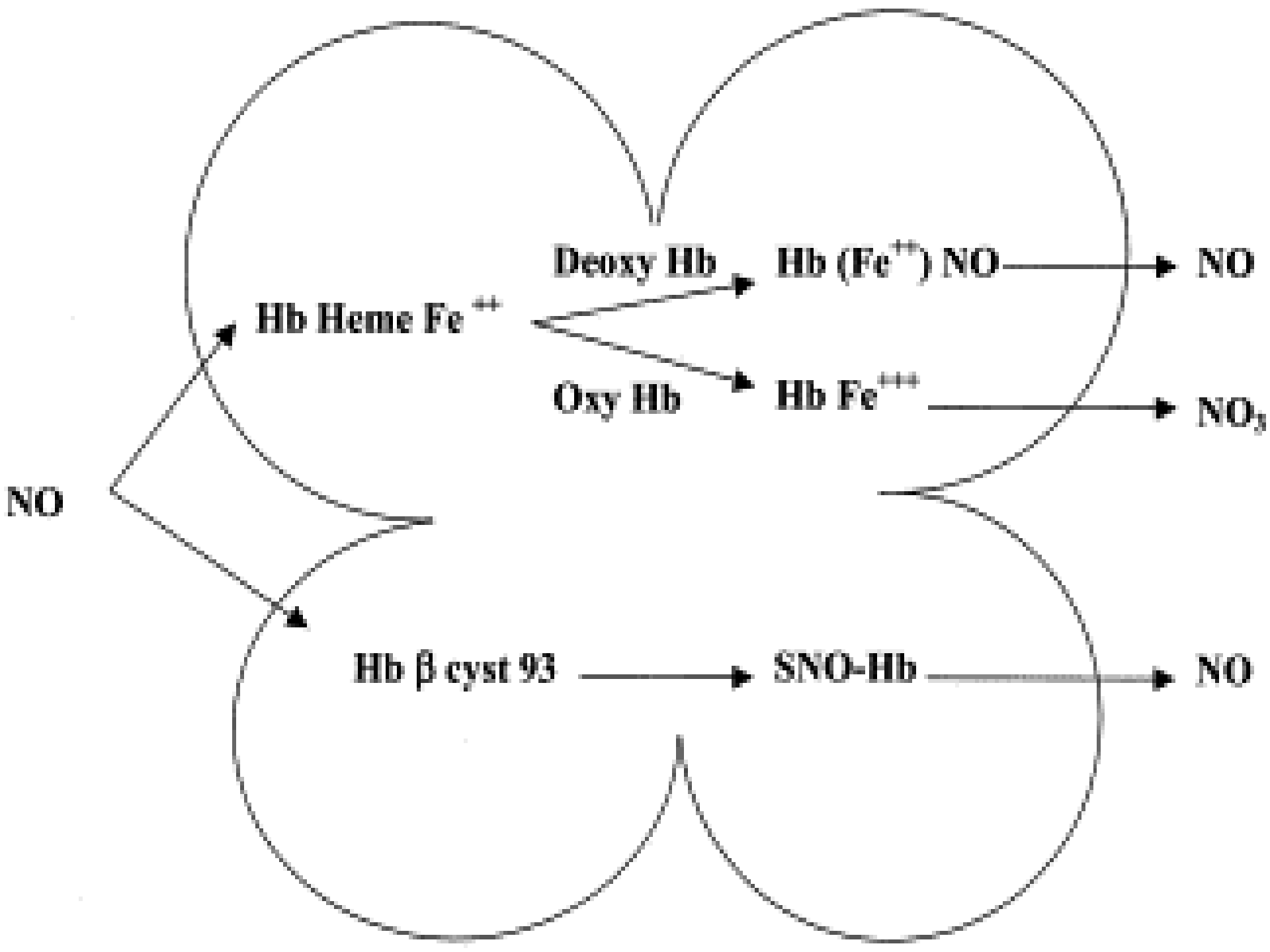
- Reflects cooperative effects of multiple oxygen binding
- Accounts for the sigmoid shape of the oxyhemoglobin dissociation curve
- Hill coefficient of adult RBC 2.7(2.4-2.9)

Oxyhemoglobin dissociation Curve

- Factors that modify oxygen binding affinity
  - 2,3 DPG, carbon dioxide, hydrogen concentrations, and body temperature
    - Binding of 2,3 DPG between Beta chains of Hb stabilizes the tense conformation and reduces affinity for oxygen
    - Decreased 2,3 DPG increased oxygen affinity—curve is shifted left



What else does Hemoglobin do?


Deoxy Hb
Hb (Fe++) NO
NO
Hb Heme Fe ++
Oxy Hb
Hb Fe+++
NO3
NO
Hb β cyst 93
SNO-Hb
NO

History of hemoglobin-based oxygen carriers

Hemolysates

- Sellards and Minot 1916-infused lysed RBCs to evaluate tolerance to hemolysis
- Amberson et. al 1933—First in vivo studies
  - Bovine hemolysate exchange transfusions in cats and dogs
  - Intact neurologic function after transfusion
  - Cats able to land on feet when dropped upside down
  - Animals died in 5 to 6 hours

Hemolysates

- Amberson et al 1949
  - Transfused lysed RBCs into 14 patients
  - “Dissolved Hb transports oxygen much as it does when confined to the RBC
  - 1 of the 14 received 2300ml’s(250g HB) secondary to postpartum hemorrhage
  - Died day 9 due to renal failure

Human Hemolysates and Renal Dysfunction

- Brand and Miller in separate studies concluded that human hemolysates produced renal dysfunction
- Speculated cause
  - RBC membrane(stromal lipid)
- Moss 1973- evidence that stromal contamination provoked intravascular coagulation

Stroma-free Hemoglobin

- Rabiner and De Venuto 1970's
  - Crystallization as a purification method
- Savitsky 1978
  - Infused 250 ml's of 99% stromal free hemoglobin into 8 healthy volunteers
    - Transient renal dysfunction
    - HTN
    - Abdominal pain

Issues to Overcome

- Spontaneous dissociation into dimers and monomers
- Increased oxygen affinity, P_{50}-12_{mmHG}
- Short intravascular retention
- Abnormal colloid osmotic pressure

1980’s

Approaches to Improve Oxygen Affinity

- Stabilize the Deoxy-HB
- Internal Cross link
- Recombinant DNA mutagenesis
- Use Non-human hemoglobin

Approaches to Improve Vascular Retention

- Internal cross link of Hb tetramers
- DNA mutagenesis
- Surface conjugation
- Liposomal encapsulation
- Polymerization of HB tetramers

First Attempt

- DCLHb—Diaspirin cross linked Hb

Phase III Clinical Trial- 1990's

- Compared 1000ml DCLHb (1000g Hb) vs. Normal Saline as the initial resuscitation agent in patients presenting to the hospital in acute hemorrhagic shock
- Hypothesis—DCLHb administration during the first 2 hours of in hospital resuscitation could reduce the 28 day mortality

» Sloan, Koenigsburg, Gens, et al. JAMA 1999.

WHY???

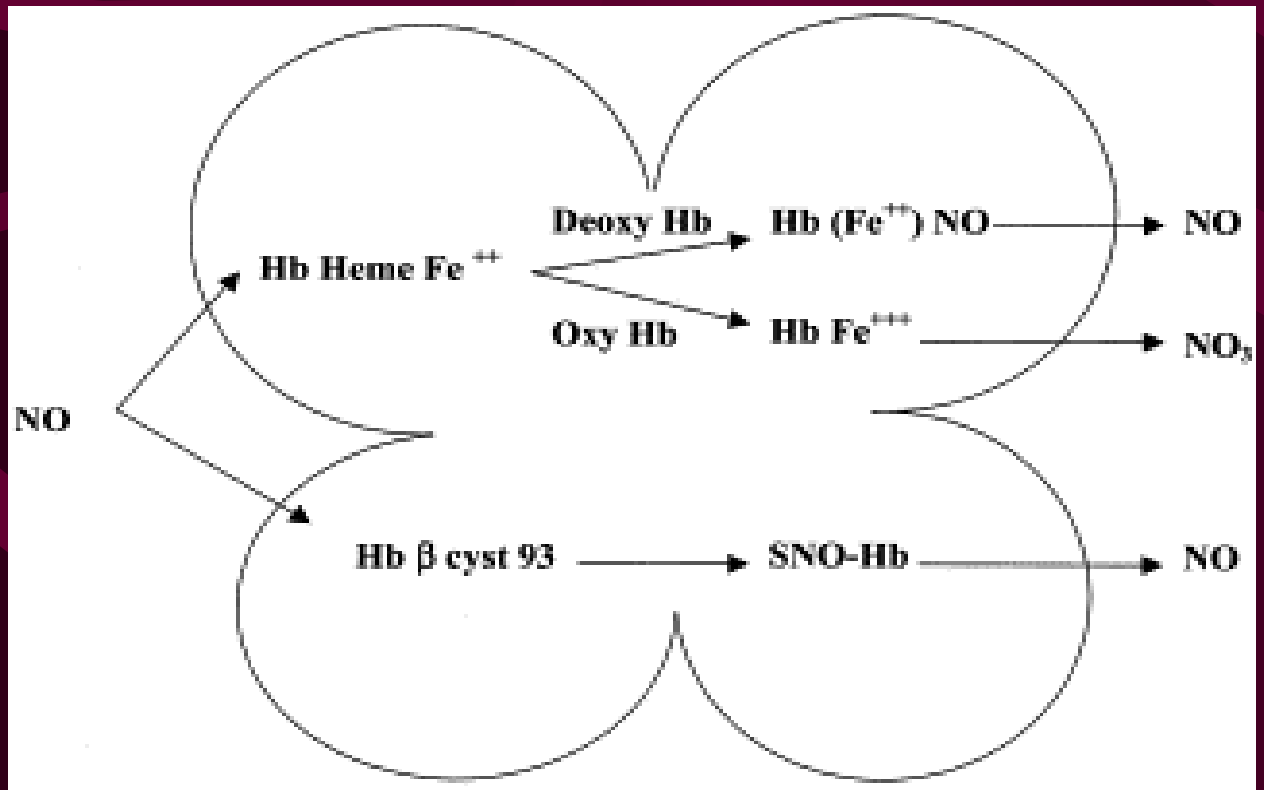
- Decreased oxygen delivery secondary to increased vascular resistence
- Macro and Micro vascular effects

What?

- 1993 Hess et al. @ LAIR—DCLHb doubled SVR and PVR, associated drop in Cardiac Index (CI)
- DCLHb in critically ill patients reduced Norepinephrine requirements until @ 7.5 hrs PVR increased and CI, O_2 delivery decreased. Reah et al. *Crit Care Med* 1997.

Mechanism

- Nitric Oxide (NO) Scavenging
- Remember the clover looking slide



That Can’t be it

- Oncotic effects—Vascular expansion decreases viscosity, Endothelial shear forces decrease, NO release is decreased.
- Pre-capillary arterioles are sensitive to hyperoxia. McCarthy, et al. *Biophys Chem* 2001.
- Endothelin release

Remember

- It’s all about oxygen delivery
- Oxygen supply and demand are coupled under standard operating conditions
- Macro and Micro circulatory effects are interconnected

Present Status

- Currently successful oxygen carriers are polymerized
  - Increased intravascular retention
  - Reduced colloid osmotic pressure
  - Attenuation of vasoconstriction

Current Lineup

Product	Manufacturer	Hb Source	Polymerization
Hemopure	Biopure Corp	Bovine	Glutaraldehyde
PolyHeme	Northfield Lab	Human	Glutaraldehyde

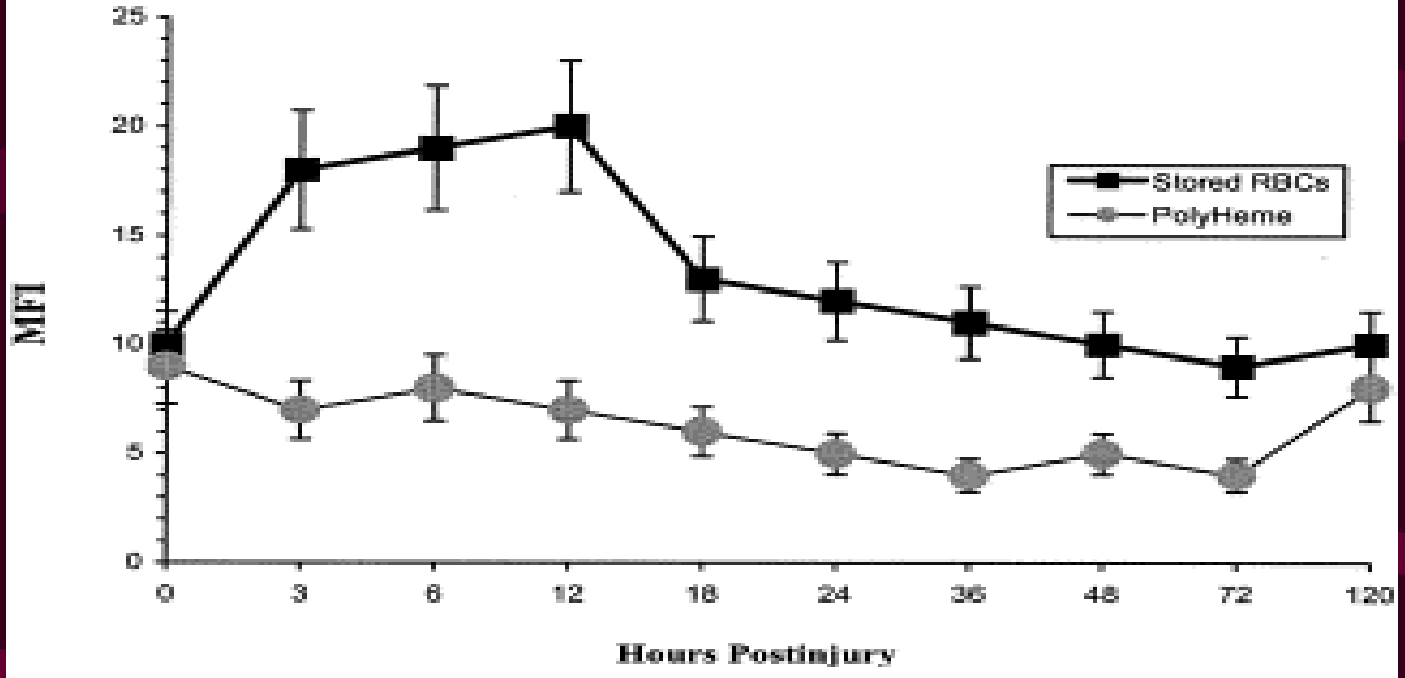
	Hemopure	PolyHeme
P_{50} (mm Hg)	38	29
Half-Life	19 hours	24 hours
Shelf-life @ 21 C	>2 years	> 6 weeks

PolyHeme

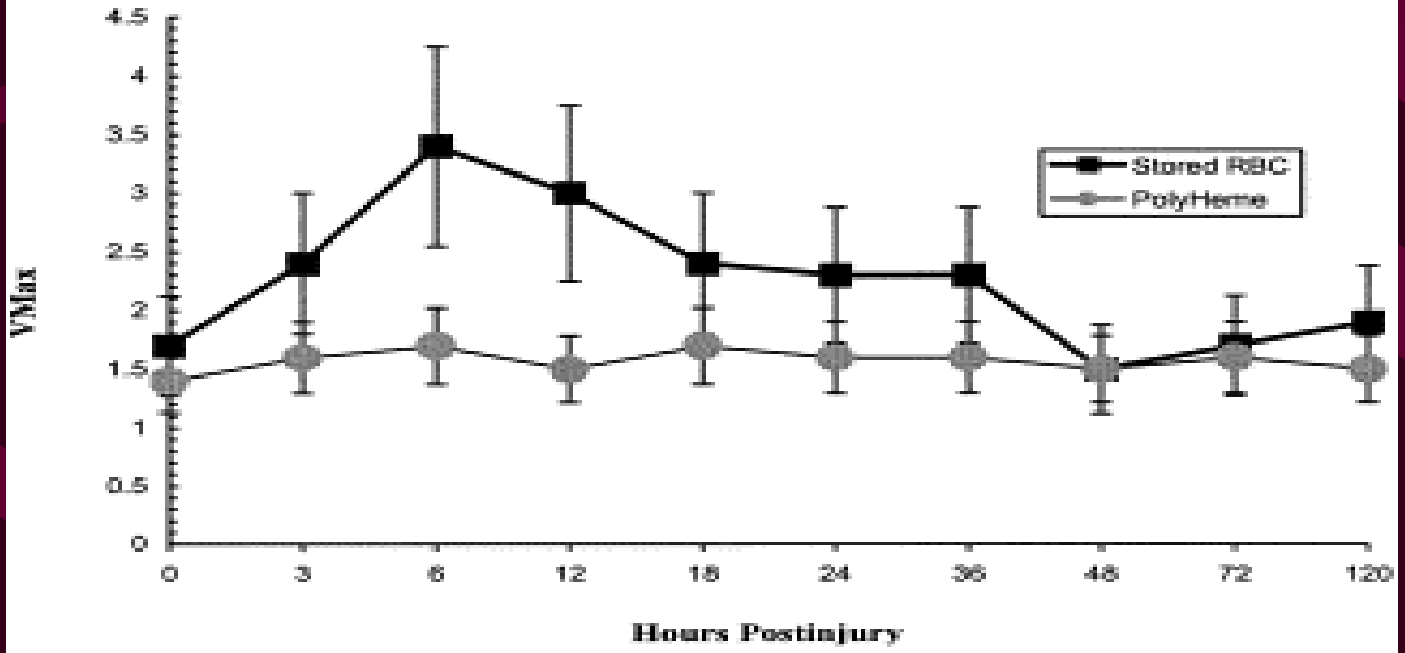
- Case Reports of efficacy
- Safety established in healthy volunteers

PolyHeme in initial Resuscitation

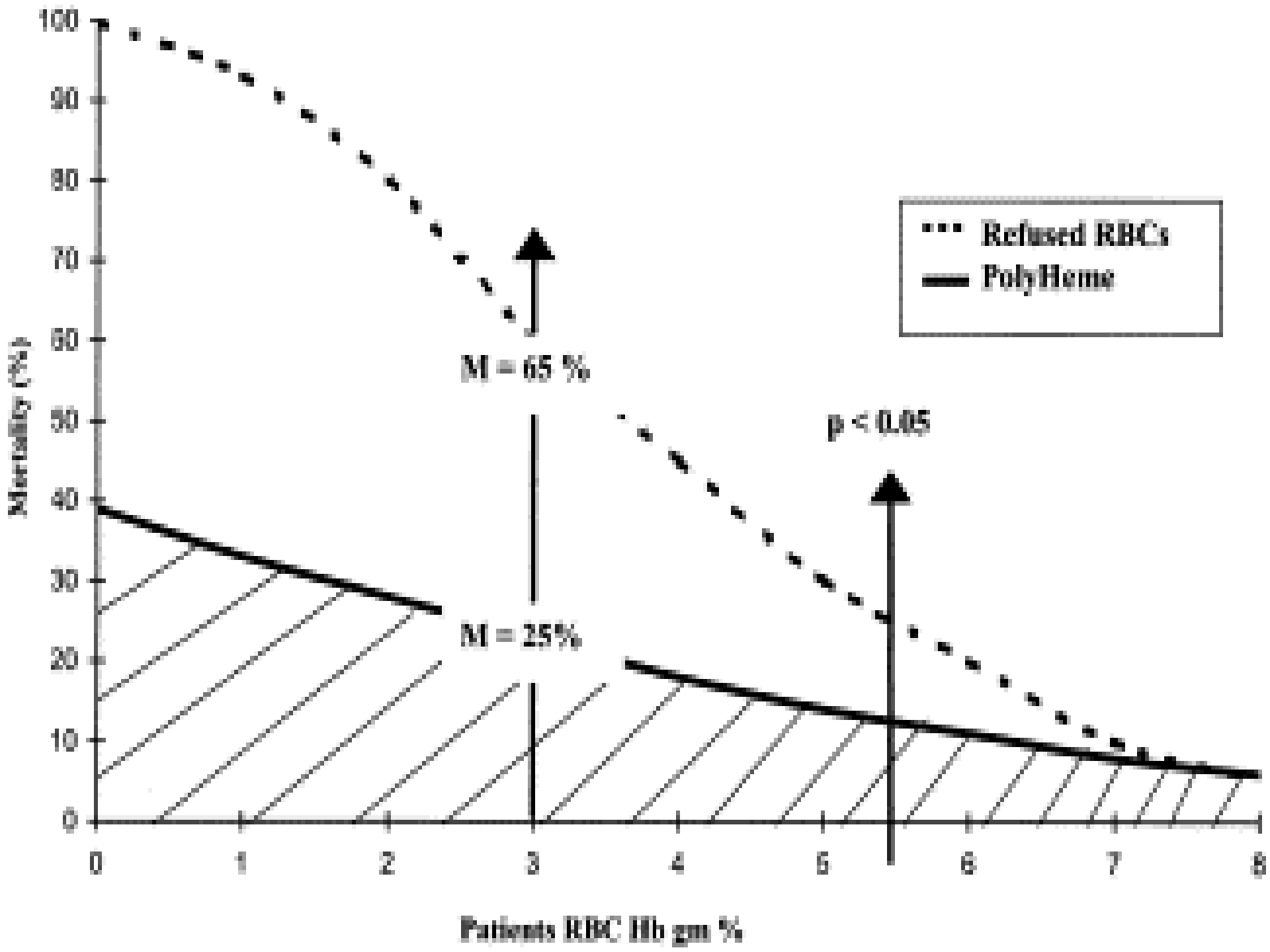
- 39 patients received 1(n=14), 2 (n=2), 3(n=15), or 6(n=8) units
- Patients were randomized to either PRBC's or PolyHeme.
- Map, Pap, CI, and PAWP were measured every 4 hours post infusion.
- No statistical differences were noted


MFI
25
20
15
10
5
0
0
3
6
12
18
24
36
48
72
120
Hours Postinjury
Stored RBCs
PolyHeme

A


VMax
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
3
6
12
18
24
36
48
72
120
Hours Postinjury
Stored RBC
PolyHeme

B


Refused RBCs
PolyHeme
M = 65 %
M = 25%
p < 0.05
Mortality (%)
Patients RBC Hb gm %
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8

Our Study

- Randomized Placebo Controlled- 16 swine
- GETA
- Underwent hemorrhage (1ml/kg/min) to remove 50% of EBV in 3 increments (10%, 30%, 50%)

- Resuscitated with equal volume of HBOC201 or oncotically matched HSA
- Molecular Probes Fluorosphere microspheres used to determine regional blood flows
- Blood and tissue samples obtained 2 hours after final hemorrhage

Baseline Stats

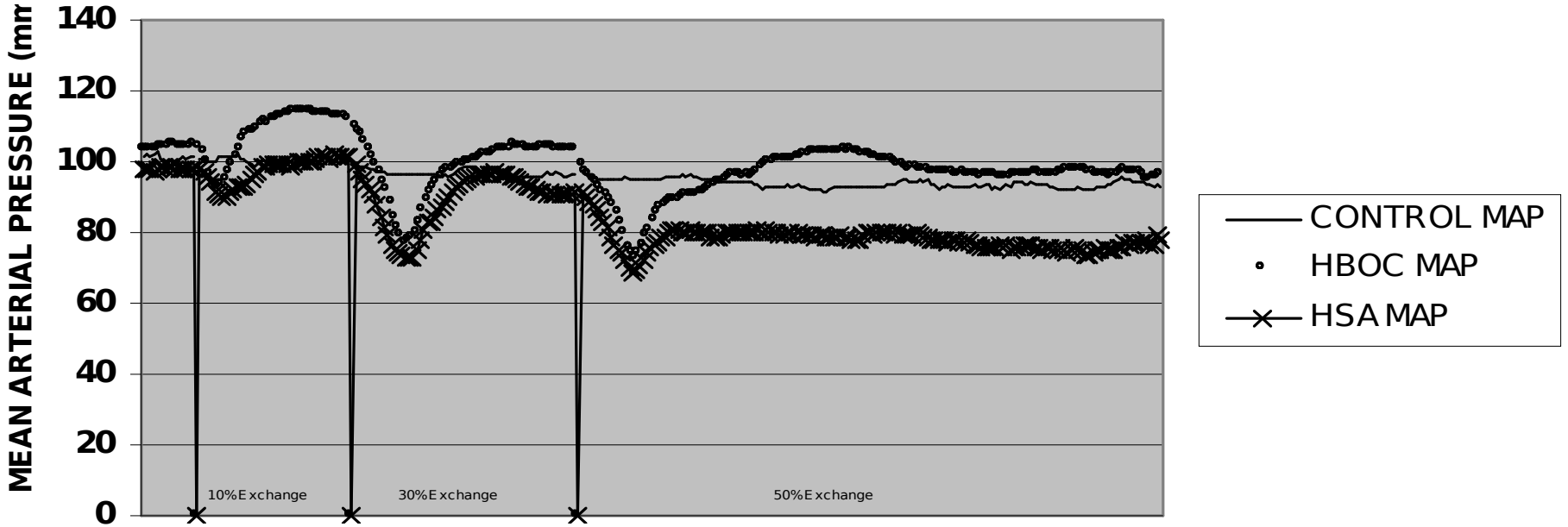
	Weight Kg	BSA cm^2	Crystalloid ml	Exchange Volume ml
HBOC201	27.7± 1.8	83.1± 4.2	147± 83	981± 74
5.9% HSA	28.4± 2.3	83.7± 4.4	115± 72	998± 80

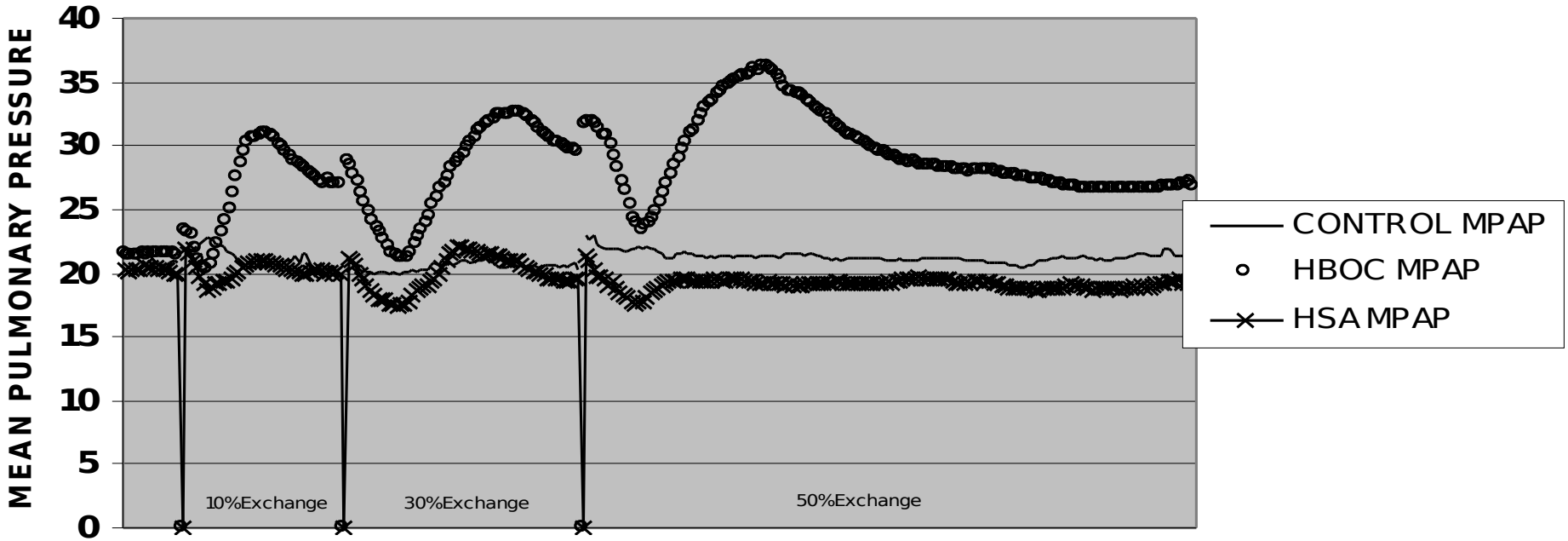
Baseline Hemodynamics

		MAP mmHG	CVP mmHG	LVEDP mmHG	CI $L \cdot min^{-1} \cdot m^{-2}$	HR bpm	MPAP mmHG	PVRI dynes•/cm^{5}• m^{2}	SVRI dynes•/cm^{5}• m^{2}
Baseline	HBOC	104± 8	6± 2	5± 1	3.9± 0.5	98± 16	22± 3	343± 62	2110± 493
	5.9% HSA	98 ± 8	5± 2	5± 2	4.1± 0.5	96± 11	21± 3	330± 62	1991± 391

50% Exchange Hemodynamics

		MAP mmHG	CVP mmHG	LVEDP mmHG	CI $L \cdot min^{-1} \cdot m^{-2}$	HR bpm	MPAP mmHG	PVRI dynes•/cm^{5}•m^{2}	SVRI dynes•/cm^{5}•m^{2}
50% Exchange	HBOC	98±13	5±3	6± 3	3.6±0.5	90±14	**28±4***	**521±101***	1891±103
	5.9% HSA	**77±18***	4±2	3± 2	**5.2±0.7***	98±14	20±3	**250±35***	**1072±366***

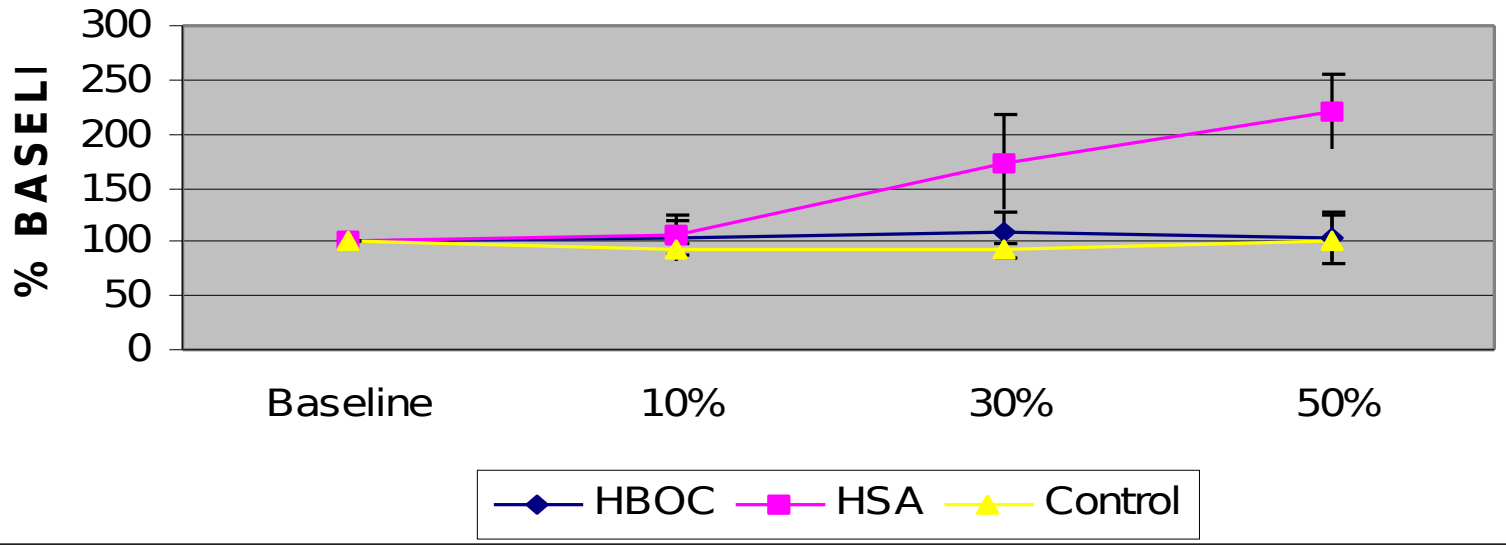

MEAN ARTERIAL PRESSURE (mm
140
120
100
80
60
40
20
0
10%Exchange
30%Exchange
50%Exchange
CONTROL MAP
HBOC MAP
HSA MAP


MEAN PULMONARY PRESSURE
40
35
30
25
20
15
10
5
0
10%Exchange
30%Exchange
50%Exchange
CONTROL MPAP
HBOC MPAP
HSA MPAP

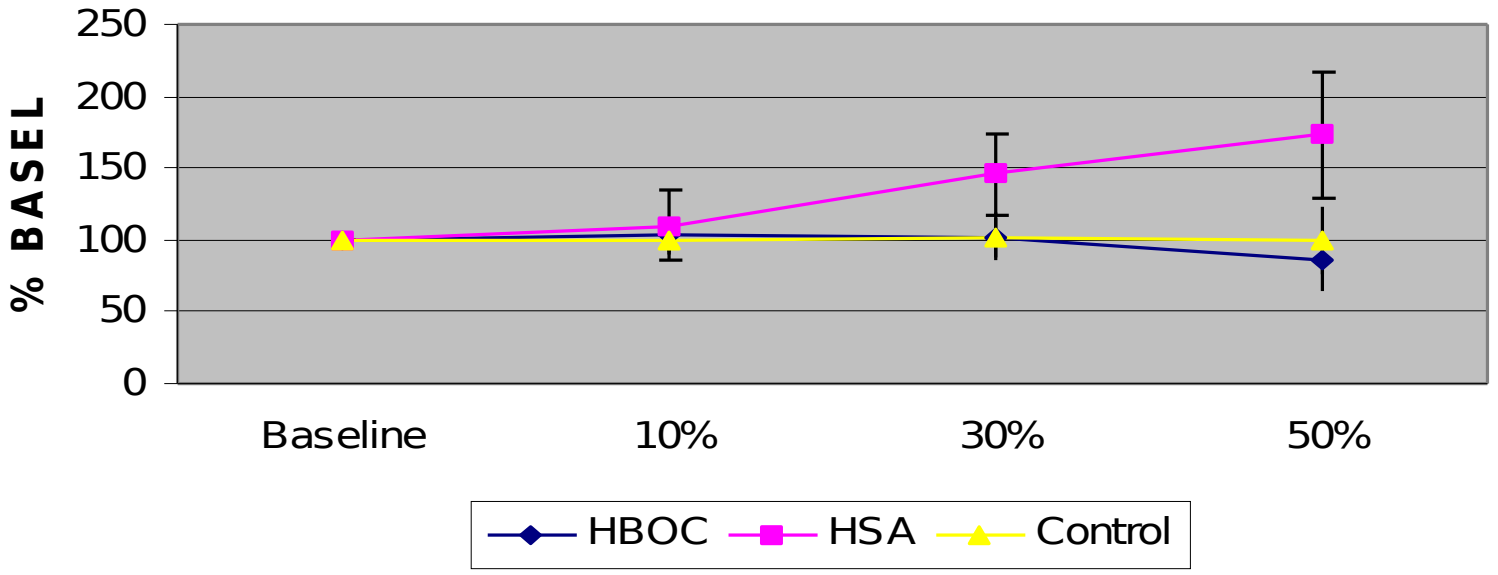
Regional Bloodflow

Units (ml/100g/min)	Left Ventricle (LV) Baseline	Left Ventricle 50% Exchange	Frontal Cortex Baseline	Frontal Cortex 50% Exchange
Control	98.0 ± 9.0	100.2±10.6	47.7± 10.8	46.8 ± 10.4
HBOC201	110.1± 14.2	90.9 ± 13.1	51.2± 10.3	52.8 ± 10.3
5.9% HSA	117.8± 25.9	180.3± 46.7	48.7± 7.2	106.3± 14

FRONTAL CORTEX



SEPTUM



Oxygen Delivery

		Arterial content ml/dl	Venous Content ml/dl	AV content Difference ml/dl	Arterial Oxygen Delivery ml/kg/min	Oxygen Consumption ml/kg/min
Baseline	HBOC201	12.7± 1.6	7.6± 1.8	5.1± 0.7	14.7± 1.9	5.9± 0.5
	5.9% HSA	12.3± 0.6	7.5± 0.8	4.7± 0.8	14.9± 0.8	5.7± 0.7
50% Exchange	HBOC201	11.7± 0.9	5.3± 0.7	**6.4± 1.2***	12.6± 1.8	6.5± 0.6
	5.9% HSA	**6.7± 0.5***	**2.7± 0.5***	**4.0± 0.4***	**10.6± 1.4***	6.2± 0.8

Discussion

- HBOC 201 was effective in restoring systemic pressure and oxygen delivery after acute incremental hemorrhage
- The pulmonary vasoconstrictive properties were significantly less than earlier compounds
- HBOC201 caused measurable alterations in systemic(minimal) and pulmonary(modest) pressures
- Pressure increases did not affect cardiac index or global oxygen delivery
- Flow studies and oxygen delivery/consumption data suggest physiologic coupling was not impaired by local vasoconstriction

Hemoglobin based oxygen carriers are on their way.

Get ready for a new tool.

Questions?

Katz LM, et al: Resuscitation 2002; 54: 77-87

Malhotra AK, et al: J Trauma 2003; 54: 915-24

Sampson JB, et al: J Trauma 2003; 55: 747-54

Meisner FG, et al: Crit Care Med 2001; 29: 829-38

Standl TG, et al: Intensive Care Med 1997; 23: 865-872

Gould SA, et al: J Trauma 1997; 43:325-32

De Venuto, et al: Transfusion 1977;17:555-6.

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